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# TECHNICAL MANUSCRIPT 260

# MICROBIOLOGICAL BARRIER TECHNIQUES

G. Briggs Phillips

DECEMBER 1965

# UNITED STATES ARMY
# BIOLOGICAL LABORATORIES
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U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

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Industrial Health and Safety Division
DIRECTORATE OF INDUSTRIAL HEALTH AND SAFETY

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## ABSTRACT

Microbiological barriers prevent the migration of microbial contami-
nants. Historically, the use of barriers in laboratory operations was
documented as early as in the 19th century. In relation to the steps
normally taken to detect and control microbiological contamination, the
tests used with microbiological barrier systems include air sampling,
surface sampling, filter and air incinerator testing, and gas-tightness
testing.

Microbiological barrier systems can be classified according to purpose,
size, and degree of containment. Sterilization and decontamination agents
are used with barrier systems for initial or terminal treatment, for the
treatment of supplies and equipment moved in or out of the system, and for
the maintenance of its microbiological state during use.

Irrespective of the shape and material used for microbiological
cabinet barriers, there are certain desirable minimum features. Photo-
graphs of a number of present day microbiological barriers and barrier
systems are presented.

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## I.  INTRODUCTION

A microbiological barrier is a device or system that, to a degree, will prevent the passage or migration of microbiological contaminants.  In the case of the food container, the can, jar, or plastic bag, prevents micro-organisms from coming in contact with the food.  The plugged culture tube, on the other hand, prevents the microorganisms inside from escaping.  The purpose of this paper is to define and illustrate the types of microbiological barrier techniques and equipment that could be useful in solving space-craft sterilization problems.

The essence of any microbiological barrier is the degree of isolation that is achieved.  Isolation is the key word because that is what the barrier should provide.  But isolation is also a magnanimous concept in that absolute isolation may not be achievable and may have more philosophi- cal than practical importance.  Luckey,[1] in illustrating the difficulty of complete isolation, suggested that a small fraction of the air we breathe was in the lungs of Christ, Mohammed, and Buddha.  According to some theories absolute isolation may not exist even on a planetary level. Thus we find that it is necessary to specify the level and degree of isolation required for a microbiological barrier.  For example, we may need a barrier for the maintenance of sterility where sterility is defined as the absence of life, based on our present understanding of what life is and present state-of-the-art methods of detecting life.  One problem with regard to sterility is that it is essentially a negative quality in which the assumption of a state of sterility is derived from the negative results of microbiological tests.  This means that there can always be a question or a suspicion regarding tests showing the absence of microorganisms, although a result based on the positive recovery of microorganisms would be accepted without suspicion.

## II.  HISTORICAL ASPECTS OF MICROBIOLOGICAL BARRIERS

It is interesting to trace the early development of mechanical barriers and methods for microbiological isolation.  Bacteriological barriers such as the flasks of Schulze, in 1836, and Schwann, in 1837, did much to invalidate the theory of spontaneous generation and heterogenesis.  Tyndall's chamber that he used in 1868 to show the relationship between the light-scattering ability of aerosols and the ability of airborne organisms to initiate growth in various infusions was an example of an early microbiological barrier. The laboratory isolation apparatus used by Davaine, in 1870, Lister, in 1878, and Koch, in 1881, enabled these men to develop the pure culture techniques that put the science of bacteriology on a sound footing.  Starting in about 1885, workers interested in germ-free life developed many types of mechanical

barrier or isolation systems. These workers started by copying the apparatus of the early gas chemists. Some of the earliest barriers were nothing more than sterile flasks and bell jars used by plant physiologists during the controversy over the means by which nitrogen is fixed into plant tissue. One such apparatus was a 4-liter jug used by Berthelot[2] in an attempt to grow plants on sterile soil. Eleven years later Nuttal and Thierfelder[3] published the results of their experiments with germ-free animals using a modified bell jar. Other modified bell jar barrier systems have been used by Cohendy,[4] Balzam,[5] and others. One of the most elaborate cabinets of that era was devised by Kuster[6] for rearing germ-free goats. Kuster's germ-free cabinet was the first to use arm-length rubber gloves. It contained essentially all the features of modern-day germ-free isolators, including an entrance airlock, air supply filters, and operation at a positive pressure. This apparatus was improved by later workers such as Climsted[7] and Reyniers.[8] Today a variety of mechanical barrier apparatus is used for research with germ-free animals. These have been adequately reviewed by Luckey.[1] The three predominant types are the heavy-walled stainless steel isolator of Reyniers,[8] the thin-walled stainless steel tank of Gustafsson,[9] and the flexible plastic isolator of Trexler.[10]

## III.  THE FIVE STAGES OF CONTAMINATION CONTROL

The use of any microbiological barrier is an exercise in microbiological contamination control. As such, the five stages of microbiological contamination control developed by the Biological Contamination Control Committee of the American Association for Contamination Control are pertinent to this discussion on barriers.[11] These five stages are shown in Table 1. For adequate analysis and surveillance of microbiological barrier systems, stages 2, 4, and 5 are particularly important.

The criteria or standards for any microbiological barrier and its operation must be established (stage 2). In many cases the criteria will be that sterility be achieved and maintained. In other instances the objective may be to limit, control, or reduce the number and types of microorganisms on or in specific components during assembly and testing. For example, one such criterion might specify the number of bacterial spores allowable per unit area of surface. The criteria may also specify the allowable airborne microbial contaminants and their particle sizes. It is also necessary to specify the exact assay techniques and other tests and procedures to be used. The specific methods and number of replicates to be used in determining sterility must be indicated. Types of culture media and culture apparatus should likewise be specified.

TABLE 1.  STAGES, APPROACHES, AND TECHNIQUES OF
MICROBIOLOGICAL CONTAMINATION CONTROL

| | |
|---|---|
| Stage 1 | RECOGNIZE AND DEFINE THE PROBLEM |
| Stage 2 | ESTABLISH CONTAMINATION CONTROL CRITERIA |
| | Maximum number of organisms allowed, types of organisms, where located, how detected, and other criteria. |
| Stage 3 | EMPLOY APPROACHES AND TECHNIQUES OF CONTROL |

| Facility Design Features | Use of Containment Equipment | Management Functions | Use of Correct Techniques | Use of Sterilizing and Decontaminating Agents |
|---|---|---|---|---|

| | |
|---|---|
| Stage 4 | MICROBIOLOGICAL TESTING AND SURVEILLANCE |

| Air Sampling | Surface and Component Sampling | Physical and Chemical Tests and Measurements | Testing of Filters, Incinerators, Sewage, Water | Gas-Tightness Testing |
|---|---|---|---|---|

| | |
|---|---|
| Stage 5 | ANALYSIS OF RESULTS AND CERTIFICATION PROCEDURES |
| | Recording results, statistical tests, use tests of items, formal or informal certification. |

In the fourth stage, the specified testing and surveillance procedures are carried out.  In any barrier system one or more of these techniques is needed to assess whether the techniques employed (stage 3) achieved micro- biological control that meets the criteria established (stage 2).

A partial list of the microbiological barrier control tests in common use includes:

1) Microbial air sampling - Air impaction samplers, liquid impingers, and settling plates are used most frequently.  The results of impaction and impinger samples are given in terms of viable particles per cubic foot of air and/or microorganisms per cubic foot of air.  The results from settling plates are expressed as viable particles per square foot per hour.

2) Particle size sampling - Liquid impinger samples with pre-impingers offer some particle size selectivity. The Andersen cascade sieve sampler is frequently used to discriminate the airborne viable particles in a microbiological aerosol into six particle size ranges.

3) Surface sampling - Cotton swabs or Rodac plates are usually used. Results are expressed as microorganisms per unit area of surface.

4) Surface contamination accumulation tests - Small sterile strips of stainless steel, glass, or plastic are placed in the environment. After various exposure periods, strips are collected and assayed for viable micro-organisms. Results are usually expressed as microorganisms per square foot.

5) Component surface testing - Small components in systems under microbiological contamination control may be tested by complete immersion in an appropriate nutrient fluid or by washing the component in sterile saline that is then quantitatively assayed for viable microbes.

6) Internal testing of components - Obviously more information on methods for determining internal sterility of components is needed.

7) Special culture tests - Special microbial detection and assay tests may be devised for other materials such as oils, greases, powders, etc.

8) Filter and incinerator testing - Periodic microbiological testing of all air filters and incinerators used in contamination control systems is required. Testing must be done in such a manner that a break of sterility is not involved.

9) Gas-tightness testing - A barrier system can be evaluated for microbiological tightness by determining its gas-tightness. The ability to contain gas molecules is prima facie evidence that the system will contain microbiological particulates. Gas-tightness leak detectors include; (i) thermal conductivity detectors, (ii) combustible gas detectors, (iii) infrared absorption detectors, (iv) argon differential "sorption" detectors, (v) differential pressure transducer detectors, (vi) ultrasonic leak detectors, (vii) mass spectrometer detectors, and (viii) halogen detectors. In high-vacuum systems, the Pirani, hydrogen, and helium ionization gauge leak detectors may be used or radioactive gas detectors may be suitable. The halogen leak detector is probably the most commonly used detector because it is relatively inexpensive, rugged, reliable, simple to operate, and has a sensitivity of $1 \times 10^{-9}$ cc of gas per second. The mass spectrometer leak detector is one of the most sensitive detectors available. Units are available that can detect vacuum leaks as low as $1 \times 10^{-14}$ cc per sec. However, the mass spectrometer is expensive and is a complex instrument that requires a skilled operator as well as trained maintenance personnel.

10) Miscellaneous measurements - To insure maximum potency of the decontaminants used, it is important that chemical titrations be made and records maintained of the concentrations of chemicals such as peracetic acid, ethylene oxide, and chlorine solutions. Records of the temperatures and exposure times should be maintained when materials are treated in autoclaves or dry-heat ovens. The temperatures of air incinerators, incubators, etc. should be appropriately observed and recorded. Insofar as possible, temperature readings should be made at the most insulated or protected areas in the material being treated. Ventilation rates should be tested at regular intervals.

The control criteria that are established in stage 2 are the guidelines for the analysis of results and certification of stage 5. Moreover, it follows that corrective actions should be started when a microbiological barrier is shown to be out of control or not meeting the minimum standards.

The choice of the proper microbiological barrier and the techniques for its use depend in large part on the selection of the criterion of control. Thus, if one wished only to prevent excessive loading of spacecraft components with microbial spores a different type of barrier would be indicated than that needed if the criterion of control was the maintenance of sterility. In the latter instance only an absolute barrier system would suffice.

The control of the microorganisms in a system is also related to the ability to detect and enumerate the microbial load in the system at any particular point in time. Contamination control is achieved if the microbial load does not exceed the level established as the lowest acceptable limit. Maintenance of control, however, is complicated by the fact that the microorganisms in a population may be going through simultaneous processes of multiplication and death. Insofar as these processes are concerned, the most stable condition of microbiological control is that of sterility—the absence of all viable microorganisms.

Once a sterile barrier system is in operation and under good control, maintenance of the sterile environment during work is more of a mechanical and engineering problem than a biological one. This emphasizes the need for the proper training of personnel in work techniques that will avoid rupture or violation of the sterility barrier.

Verification of the sterility of microbiological barrier systems is a problem of some concern. Within a sterile barrier system, it is possible to employ several direct approaches to microorganism detection, such as the exposure of quantities of liquid or solid culture media or germ-free animals to the environment within the system. It must be emphasized, however, that the present state of the art is such that the ability to demonstrate the presence of viable microorganisms within cabinet systems or in spacecraft components decreases as the number of viable microorganisms becomes smaller. In spite of limitations of this sort it is significant that Trexler[12] has been able to maintain a colony of mice and a colony of rats in an apparent germ-free condition within a barrier system for more than 12 years.

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## IV. CLASSIFICATION AND DESCRIPTION OF MICROBIOLOGICAL BARRIERS

Microbiological barrier systems can be classified according to purpose, size, and degree of containment, as shown in Table 2. "Purpose" classification relates to the direction of the barrier system. Thus, germ-free animal barriers prevent contamination from entering; microbiological safety barriers prevent the escape of infectious microorganisms. The difference is sometimes illustrated by referring to "product protection" and "personnel protection" systems. Obviously, for spacecraft sterilization we are most interested in the product protection systems. Occasionally a barrier system is needed that will operate in both directions at the same time. An example of this is the barrier cabinets to be used in the Lunar Sample Receiving Laboratory. These cabinets must prevent escape of lunar material during a quarantine period, but it is also highly desirable to prevent contamination of the lunar samples with earth microorganisms.

TABLE 2.   CLASSIFICATION OF MICROBIOLOGICAL BARRIER SYSTEMS

| Classification According to | Types |
|---|---|
| Purpose | Product Protection or Personnel Protection |
| Size | Room Size       or Cabinet Size |
| Degree of containment | Absolute Barriers  or Partial Barriers |

A second method of classification refers to the size of the barrier and its position in relation to the protected environment. Is the work externalized from the worker by placement in an enclosure or cabinet of its own, or is the worker internalized within the environment and separated by protective clothing? It is possible to wrap a barrier around the work or around the worker. Wrapping a barrier around the worker is illustrated by a germ-free room entered only by people wearing sterile, ventilated plastic suits. A lesser degree of isolation would be represented by a worker in a clean room wearing a respirator and sterile garments to provide the microbiologic barrier. Some of the difficulties of internalization and in maintaining an adequate barrier around the worker are overcome by wrapping the barrier around the work. The use of hoods, cabinets, germ-free isolators, and similar enclosures illustrate the externalization type of barrier.

Finally, in classifying microbiological barrier systems according to the degree of containment, they may be described as either absolute or partial barriers. Absolute barriers allow no interchange of the protected and nonprotected environment and aim at total containment. They usually provide for placement of the material or the work to be controlled within a gastight enclosure, usually a stainless steel cabinet or a plastic isolator. Humans are separated from the system and the work is done through attached arm-length rubber gloves or by means of remote mechanical manipulators. When work within the barrier is to be protected from outside contamination, the system or enclosure is maintained at a positive air pressure. Conversely, negative pressure is used in the enclosure to prevent escape of contaminants from it. According to the criteria for micro-biological control, inlet and/or outlet air may be filtered or incinerated. Prior to use, the enclosure may be decontaminated or sterilized. Air locks, dunk baths, autoclaves, and other devices may be used to preserve the sterile integrity of the enclosure while materials are passed in and out of it.

The most comprehensive report of absolute barrier devices for personnel protection is that of Gremillion,[13] who described the gastight cabinet systems used at the U.S. Army Biological Laboratories at Fort Detrick, Frederick, Md. The systems included incubators, refrigerators, centrifuges, and balances and utilized disinfectant dunk baths and autoclaves for the entrance and exit of materials.

Flexible plastic barriers at a positive pressure for the absolute containment of germ-free animals are largely a development of Trexler.[10] Rigid plastic absolute barriers have also been used.[14] Stainless steel containers in common use with germ-free animals are similar to those originally designed by Reyniers or by Gustafsson. The types of barriers or isolators for germ-free animal experimentation have been adequately summarized by Luckey.[1]

Absolute containment can be achieved in a room-sized environment but only by the use of ventilated suits or some other enclosure for persons entering the room. A product protection room for sterile assembly work must be closed and sterilized prior to use. Suited personnel enter through a series of air locks where the outside of the suit is sterilized with chemical agents.

The sterile room concept derives primarily from germ-free research. Schottelius[15] built the first germ-free room in the center of a large empty room at the Institute of Hygiene of the University of Freiburg. This room was used in an attempt to raise germ-free chicks. Reyniers[16] described a room-sized tank 2.5 meters in diameter and 5 meters long used for rearing germ-free animals. The tank was sterilized with steam under pressure. Before entering the sterile tank the operator wearing a plastic diving suit submerged himself for 30 minutes in an entrance dunk bath

filled with 2% formaldehyde solution. Trexler[17] converted a laboratory
room into a sterile room by covering the walls and ceilings with asbestos
flexboard and covering this with polyester resin-impregnated glass cloth.
He covered the cement floor with a plastic paint. The room was chemically
sterilized before use. Workers wore plastic suits that were chemically
sterilized in an air lock as they entered the room. Luckey[1] described a
sterile building about 14 meters by 8 meters (46 by 26 feet) at the Depart-
ment of Virology, Rega Institute, University of Louvain in Belgium. This
facility apparently is used for the large-scale production of germ-free
animals.

A recent use for sterile plastic isolators of roomette size is their
application to special problems of hospital patient care.[18] Patient
isolators, or "life islands," that actually enclose the hospital bed
have been used with patients who, because of the nature of their injury,
illness, or treatment, have little resistance and high risk of severe
infection. Some room isolators are as large as 8 by 8 feet and are of
sufficient size to allow the patient to get out of bed and walk up and
down beside the bed. The plastic room isolators are gas-sterilized.
Doctors and nurses have access to the patients through attached arm-length
rubber gloves.

Absolute barrier rooms for product protection have been successfully
employed. One such room that I worked in some years ago housed a non-
tight spray-drier machine used to process hazardous biological materials.
The room had plastic-coated walls, ceilings, and floor. After the drier
had operated, sprays of peracetic acid were used to sterilize the contami-
nated room. Suited individuals working in the room sterilized the outside
of their suits as they left the room. At several infectious disease labora-
tories similar rooms, operated at a negative pressure and entered in venti-
lated suits, are used to house large animals infected with highly contagious
disease agents.

Classification of a microbiological barrier as a partial barrier indi-
cates that something less than absolute containment or isolation is to be
achieved. Open-panel ventilated cabinets and hoods, clean benches,
specific pathogen-free animal-rearing facilities, bio-clean rooms, laminar
flow benches, and laminar flow rooms are examples of barrier arrangements
that provide less than absolute microbiological isolation or containment.

Before considering specific examples of partial barriers, I should like
to emphasize the importance of the dichotomy between absolute and partial
barriers. The principal point that I wish to make is that there is no
rational basis for believing that partial barrier systems that allow
uncovered or unprotected workers within the protected environment can
ever be improved to the point where they can substitute for absolute
barriers. As long as workers in a protected environment are capable of
shedding microorganisms the barrier can hardly be called absolute. To
be more specific, I believe that the principles of minimum turbulence

air flow (incorrectly called laminar air flow) and other engineering
developments can do much to improve partial barriers, but that such
systems can never substitute for the mechanical separation of environments
provided by absolute barrier systems. There are three specific reasons
for this statement:

1) Partial barrier systems are difficult if not impossible to
design to be fail-safe.

2) Air flow patterns, even those in minimum turbulence air flow
systems, are easily disrupted by people and equipment in the path of the
air.

3) The velocity or speed of movement of people and equipment often
exceeds the velocity of the air being moved in the barrier system. Most
partial barrier systems utilize air velocities between 50 and 150 linear
feet per minute, but a person walking at the very modest speed of 2 miles
per hour is moving at 176 linear feet per minute.

The above comments are in no way intended to minimize the developments
that have taken place in the last five years in regard to clean rooms,
clean benches, and the like, but the intent is to create a clear distinc-
tion between barrier systems that are capable of maintaining sterile condi-
tions or an absolute separation of environments and those that functionally
cannot perform in this manner.

The partial barrier principle uses enclosures, cabinets, or rooms that
are not gastight and that are not completely closed. Containment depends
on an inward or an outward flow of air through an open working panel,
through open glove ports, or through or across a room area. The inlet or
outlet air or both may be filtered. For cabinets, since only the hands
and arms of the operator extend into the environment, the chance of spread-
ing contamination to or from humans is minimized. A great number and
variety of partial barrier product protection and personnel protection
devices are in use today. The chemical-fume hood finds its biological
equivalent in the inward-flow biological safety cabinet.

Microbiologists handling infectious disease agents have long realized
the need for mechanical barriers to internalize hazardous procedures.
Safety cabinets were in use in German laboratories early in the century.[19]
Shepard, May, and Topping[20] at the National Institutes of Health developed
a wooden cabinet for hazardous laboratory operations. In England, Van den
Ende[21] developed similar cabinets for use during large-scale production of
scrub typhus vaccine. The first stainless steel microbiological barriers
for infectious disease work were described by Wedum.[22] Other types and
improvements have been described by Reitman and Wedum,[23] Phillips et al.,[24]
Gremillion,[13] Blickman and Lanahan,[25] Wedum and Phillips,[26] and Phillips.[27]

Cabinets with outward flow for the dust-free assembly and packaging of components undoubtedly reduce microbial contaminants. In the pharmaceutical field, such cabinets are frequently used during the filling and packaging of biologicals.

The principles of the construction and operation of sterile rooms have been modified and slightly downgraded for partial microbiological barriers. Animal breeders have been able to improve the quality of laboratory research animals by closed-colony techniques wherein all animals derive from a disease-free nucleus breeding colony that is maintained in a thoroughly decontaminated room. All supplies and materials entering the room are sterilized and personnel working in the room wear sterile garments and respirators. A slightly different use for room-sized barriers is that for holding infected experimental animals the size of monkeys and larger. In this case such rooms are made essentially air-tight and are maintained at a negative air pressure. Entrance to the room is restricted to personnel wearing ventilated suits that are chemically disinfected before leaving the room. These animal rooms are treated with gaseous germicides after each experiment.

The clean room may also be classified as a type of microbiological barrier, although it is obvious that the presence of unsuited human occupants limits the degree of microbiological isolation that can be achieved. Applying the principles of minimum turbulence air flow has improved the degree of isolation possible in clean rooms but, as pointed out above, the human in the system still represents an unpredictable variable and makes microbiological isolation impossible to achieve.

## V.   STERILIZATION AND DECONTAMINATION OF MICROBIOLOGICAL BARRIERS

We have previously defined sterilization as that negative state in which the absence of life is indicated by the failure of the test procedures to produce a positive result. The agent bringing about this condition is a sterilizing agent. For the purpose of the following discussion we also identify a decontaminating agent as one that is effective in destroying or eliminating microbiological contamination but not necessarily to the degree of producing sterility.

Sterilization and decontamination agents have three general uses in microbiological barrier systems. Table 3 shows recommended agents for each barrier application. For sterile assembly procedures or those where protection of the product or operation is desired, the barrier system and all of its components should be sterilized or decontaminated before use. For barrier systems to protect the operator, as in the case of infectious disease laboratory work, decontamination would follow rather than precede use of the barrier.

The second use relates to the treatment of supplies and equipment that must be moved in or out of the barrier while it is in use. The third use relates to decontaminating procedures used within the barrier while it is in operation to maintain its sterility or microbiological state. It is obvious that not all the sterilizing and decontaminating agents shown in Table 3 will act with equal efficiency and reliability. All have some advantages and disadvantages. The following discussion covers pertinent points concerning sterilization and decontamination agents classified under four main headings: heat, vapors and gases, liquid decontaminants, and radiation.

TABLE 3.  STERILIZATION AND DECONTAMINATION AGENTS
FOR USE IN MICROBIOLOGICAL BARRIERS

| Use of Sterilization or Decontamination Agents | Recommended Sterilizing or Decontaminating Agents |
|---|---|
| Sterilization or decontamination of barrier systems before use | 1. Steam under pressure<br>2. Ethylene oxide gas<br>3. Peracetic acid<br>4. Steam formaldehyde<br>5. Beta-propiolactone |
| Treatment of supplies and equipment moved in or out of barriers | 1. Steam under pressure<br>2. Dry heat<br>3. Ethylene oxide gas<br>4. Dunk bath solutions<br>5. Peracetic acid pass-through<br>6. Ultraviolet air lock |
| Maintenance of microbiological conditions inside barrier during its use | 1. Atmosphere of germicidal gas<br>2. Irradiation with ultraviolet<br>3. Periodic wash-down with liquid decontaminants |

A.  HEAT

Heat is the most effective and reliable method of inactivating micro-organisms and should be used whenever possible. The exposure temperatures, and times required for sterility are known and can be readily controlled. Dry-heat ovens containing air or an inert gas can be used while passing

some materials and supplies in and out of sterile barrier systems. Steam
sterilizers also are recommended for passing materials in and out of barrier
systems; moist heat is faster and more reliable. Except for relatively
small chambers, heat sterilization of entire barrier systems is usually
not possible.

## B. VAPORS AND GASES

Ethylene oxide,[28] formaldehyde,[29] beta-propiolactone,[30] and peracetic
acid[31] are chemicals used in gaseous, vapor, or fog form. When these chemi-
cals are employed in closed systems and under controlled conditions, excel-
lent decontamination and even sterility can result. However, the properties
and limitations of each should be thoroughly understood in relation to the
barrier system.

Ethylene oxide is a highly penetrating and effective sterilizing gas,
convenient to use, versatile, noncorrosive, and effective at room tempera-
ture. However, the gas is slow in killing microorganisms and must be mixed
with other gases to avoid explosion hazards. Ethylene oxide is widely used
to treat many items not suitable for heat sterilization. It has been used
in mixtures with carbon dioxide or nitrogen, which requires that it be used
under pressure. Its most extensive use today is in the form of a low-
pressure mixture with chlorofluorohydrocarbons (freons) in disposable cans
or cylinders.* In this form it is a highly practical and convenient tool
for increasing the usefulness of the laboratory autoclave. A steam auto-
clave can be inexpensively converted to its use without interfering with
the use of the autoclave with steam. A definite limitation to the use of
ethylene oxide is the required exposure time. In concentrations practical
for use, a minimum of 6 hours is required to sterilize materials contami-
nated with bacterial spores. Longer (overnight) exposures are recommended
for routine use. Another limitation is that neoprene gloves, clothing,
footwear, or other plastic, rubber, or leather wearing apparel that have
been treated with ethylene oxide must be thoroughly aired for 24 hours
before use because of the irritating action of absorbed ethylene oxide
on human tissues. Ethylene oxide gas mixtures can be used to sterilize
microbiological barriers prior to use or to treat certain materials passed
in or out of the barrier.

Almost any method of dispersing formaldehyde into the air in suitable
quantities is satisfactory for the use of this chemical as a space decon-
taminant. Because it is most efficient at higher temperatures and humidi-
ties, steam ejectors or steam vaporizers are most conveniently used for
small areas. Although formaldehyde has a rather irritating odor, it is
relatively noncorrosive to metals, and it can be generally assumed that

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* Ethylene Oxide Mixture, Pennsylvania Engineering Co., Philadelphia,
  Pennsylvania.

any equipment or apparatus that will not be damaged by the necessary humidity will not be damaged by the formaldehyde. For decontaminating room-sized barriers, mechanical vaporizers are used.* The formaldehyde solution is introduced in a concentration of one milliliter per cubic foot of space. In making this calculation, any airflow through the space must be taken into account, and additional formaldehyde added to obtain the above concentration. A hold period of 8 to 10 hours is recommended for room-sized barriers. Formaldehyde fumes are persistent, and a room may require two to three water washes of the floor and 2 to 3 days' ventilation before normal entry.

For smaller ventilated and closed barrier systems such as gastight cabinets, the formaldehyde solution is vaporized at a rate of one milliliter per cubic foot of airflow, plus one milliliter for each cubic foot of space within the barrier. Thus, if the barrier contains 50 cubic feet and the airflow is 20 cubic feet per minute (cfm), then 650 milliliters of formaldehyde solution (20 x 1 x 30 + 50) must be vaporized in 30 minutes. In small barriers the formaldehyde may be vaporized with a steam ejector** or a mechanical vaporizer. In the latter case care must be taken to raise the humidity by boiling water or by injecting steam into the barrier. This technique will decontaminate the entire barrier system, i.e., cabinet, exhaust filter, exhaust duct, and blower.

Beta-propiolactone (BPL) has several advantages over formaldehyde as a vapor disinfectant:

1) Its vapors are lachrymatory but less irritating than those of formaldehyde.

2) It does not readily polymerize on surfaces so that there is little or no residue.

3) It acts more rapidly. However, in the liquid state, beta-propiolactone is more toxic than formaldehyde, and in handling it, care must be taken that it does not contact the skin.

The technique for disseminating beta-propiolactone is similar to that for formaldehyde. However, more care must be exercised to make certain that the BPL is well vaporized. The chemical must leave the disseminator as a vapor or in particles small enough so that they vaporize rapidly. Otherwise, the liquid droplets settle or impinge on surfaces and dissemination is not effective. Liquid beta-propiolactone is injurious to rubber

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* Challenger Model 5100 Vaporizer, Z & W Mfg. Corp., Wickliffe, Ohio.
** Penberthy X6-96, series 1, steam ejector, Penberthy Injector Co., Detroit, Mich.

items and painted surfaces if it is not immediately washed off. After a
hold period of 2 to 3 hours, ventilation of the barrier may be resumed.
At this point, treated areas should be entered only with protective cloth-
ing and respiratory protection. Proper airing will generally allow normal
entry after 2 to 3 hours.

Peracetic acid is an excellent bactericide. As a 2% solution it can be
sprayed as a fog to decontaminate enclosures or other areas. Because
peracetic acid is extremely corrosive, it should be used in contact only
with plastics, plastic-coated materials, or stainless steel. Its wide use
for the treatment of isolators used in the rearing of germ-free animals
is adequate proof of its sterilizing ability.

## C.  LIQUID DECONTAMINANTS

There are many misconceptions concerning the use of liquid decontami-
nants. This is largely due to a characteristic capacity of such liquids
to perform well in the test tube and to fail in a practical situation where
such factors as temperature, contact, pH, concentration, and the presence
of organic material at the site of application are not considered or con-
trolled. Small variations in these factors may make large differences in
germicidal effectiveness. Hundreds of decontaminants are available under
a variety of trade names. Most may be classified as halogens, acids or
alkalies, heavy metal salts, quaternary ammonium compounds, phenolic com-
pounds, aldehydic compounds, and other organic preparations. None is
equally useful or effective under all conditions and only a few are
effective against bacterial spores.

Liquid decontaminants serve in barrier systems as the fluid for dunk
baths and as germicides for periodic washing of surfaces and items within
microbiological barriers. For dunk baths it is important to use solutions
such as formalin or sodium hypochlorite that are active against bacterial
spores. Even though many decontaminating agents are not sporicidal, some,
especially those with a reduced surface tension, will do much to lower the
microbial count on surfaces.

## D.  RADIATION

When used correctly, germicidal ultraviolet (UV) radiation is an
effective means of decontaminating air and surfaces. It can be used in
transfer air locks and within microbiological barriers. But proper use of
UV as a decontaminating agent requires an understanding of its limitations.
The radiation has limited penetrating power and thus is effective only on
exposed surfaces or in air. Proper concentration, contact time, and main-
tenance are also critical. Phillips and Hanel[32] have adequately described
the use of UV for practical decontamination applications.

Table 4 summarizes the recommended conditions of use for the steriliza-
tion and decontamination agents most commonly used with microbiological
barriers.

TABLE 4.  RECOMMENDED CONDITIONS OF USE FOR STERILIZATION
AND DECONTAMINATION AGENTS IN MICROBIOLOGICAL BARRIERS

| Sterilization or Decontamination Agents | Condition of Use[a] (temperature, concentration, exposure time, etc.) |
|---|---|
| Moist heat (autoclave, high vacuum) | 127 C, 2-3 minutes[b] |
| Moist heat (autoclave, no vacuum) | 121 C, 15-30 minutes[b] |
| Dry heat | 160 C, 2 hours[b] |
| Ethylene oxide gas | 25 C, 300 mg/l, 8-16 hours, 30% RH |
| Peracetic acid spray | 25 C, 2% with 0.1% surfactant, continuous for 20 minutes |
| Steam formaldehyde vapor | 25 C, 1 ml per cubic foot in air with RH above 80%, 30 minutes (cabinets) or 10 hours (rooms) |
| Beta-propiolactone vapor | 25 C, 200 mg per cubic foot in air with RH above 70%, 30 minutes (cabinets) or 2 hours (rooms) |
| Dunk-bath formalin (37% HCHO) | 25 C, 10%, 10 minutes |
| Sodium hypochlorite solutions | 25 C, 500-5000 ppm with 1% surfactant, 5 minutes |
| Quaternary solutions[c] | 25 C, 0.1% - 0.5%, 1 minute |

a.  Based on maximum effectiveness against bacterial spores.
b.  Not including come-up time.
c.  Not sporicidal but good cleaning agents.

## VI.  DESIRABLE FEATURES OF MICROBIOLOGICAL BARRIERS

Irrespective of the shape and material used for microbiological cabinet barriers, there are certain desirable minimum features:

1) Proper air flow or air balance within the barrier. For an open-panel barrier, this means low turbulence of inward or outward flow of air sufficient to prevent the entrance or escape of airborne particulates. The minimum air velocity is usually 100 linear feet per minute. For a closed, gastight barrier, this means operation at a positive or negative air pressure of 0.5 to 1.0 inch of water. Internal ventilation patterns for gastight barriers should be determined by the nature of the operation. Some procedures may require ventilation with inert gases or accurate control of temperature and humidity within the barrier.

2) An efficient means of sterilizing or decontaminating all interior surfaces of the system.

3) Appropriate filters or incinerators for the air supply or exhaust or both.

4) A glass or clear plastic viewing panel between the operator and the operation.

5) Internal surfaces that are resistant to chemical corrosion and free of cracks or crevices that would interfere with sterilization and decontamination.

6) Proper arrangement for handling materials within the barrier. For gastight cabinets, this means attached arm-length neoprene gloves. For open-front cabinets, a panel should be available to close the unit during decontamination. A detachable front panel containing ports for arm-length gloves ideally serves both types of cabinets.

7) Appropriate air locks, dunk baths, autoclaves, gas chambers, and other devices attached to gastight barriers so as to allow passage of essential supplies and materials.

8) Ample amount and arrangement of working space within the barrier to minimize the need to transfer material in and out of the barrier before completion of an operation.

9) Appropriate services such as electricity, gas, vacuum, air, light, ultraviolet irradiation, water, and drains.

Many of the above features also apply for room-sized barrier units.

## VII. EXAMPLES OF MICROBIOLOGICAL BARRIERS

Figures 1 and 2 show microbiological barriers made of flexible 20-mil polyvinyl chloride sheeting supported by an outer frame of aluminum or wood. These cabinets are equipped with air locks with zipper closures and tubes for attaching air inlet and outlet filters. Ventilation is achieved by attaching the cabinet into a laboratory vacuum or compressed air line. Also, an atmosphere of inert gas can easily be admitted to the cabinets via the tubes on the side.

Figure 3 shows an isolator for germ-free animals made of flexible polyvinyl chloride film. This chamber is operated at a positive pressure with the incoming air sterilized by passage through multiple layers of FG-50 spun glass.

Figure 4 illustrates the adaptability of flexible plastic for microbiological barriers. Here a small enclosure has been adapted for an operation requiring the use of a binocular microscope. Ports for gloves are provided on each side of the enclosure and a small zipper air lock is located on the left side for passing materials. A connection for ventilation can be seen at the lower left.

Figure 5 shows a somewhat larger microscope enclosure that was ventilated by attaching it to the glove port of a stainless steel ventilated cabinet.

Figure 6 again illustrates the adaptability of a plastic enclosure. In this case an enclosure has been formed around an animal cage rack and attached by tunnel to a stainless steel cabinet.

Figure 7 shows the "life island" concept that utilizes an inflated flexible plastic chamber around the bed of a hospital patient. Arm-length rubber gloves are provided for the nurses and doctors in treating the isolated patient.

The use of polyvinyl chloride or other flexible sheeting provides barriers that are economical and can be constructed in almost any size and shape in minimum time. However, these plastic barriers have the disadvantage that they can be easily punctured. Therefore, in general, when the penalty, in terms of dollars or time, for the failure of a barrier system is high, the use of flexible plastics is not recommended.

Figure 8 shows a microbiological barrier made of rigid plastic material, in this case plexiglass. Enclosures of this type are not easily punctured but the plastics used are easily scratched and often lack resistance to ultraviolet radiation, heat, and other environmental factors.
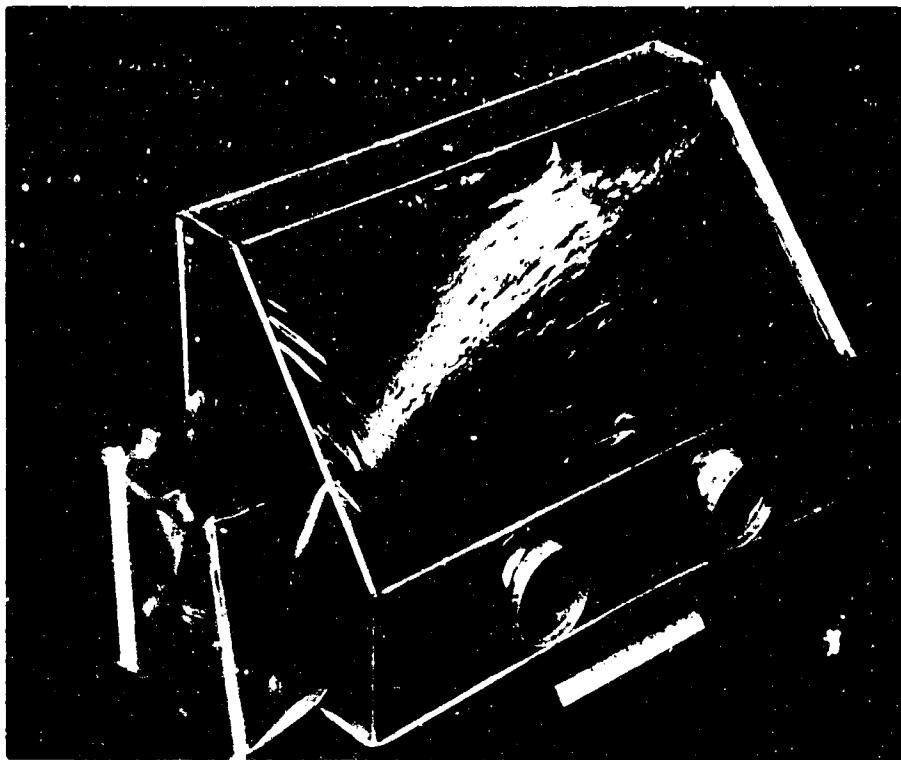
24



Figure 1.  Cabinet Made of Flexible Plastic and Supported
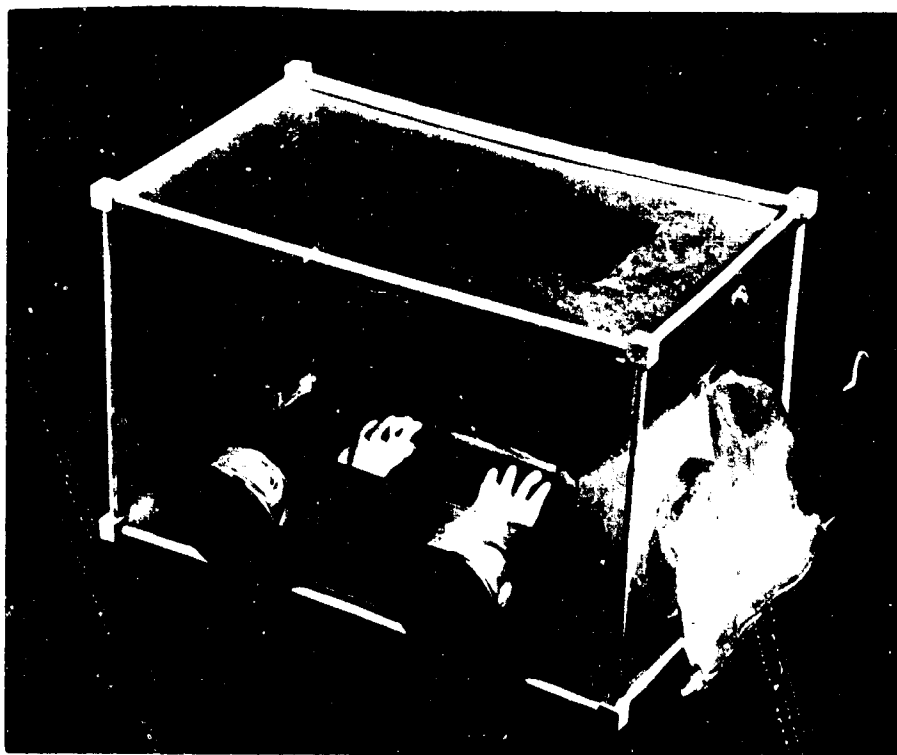by Aluminum Tubing.  (FD Neg C-1930)



Figure 2.  Cabinet Supported by Wooden Doweling.
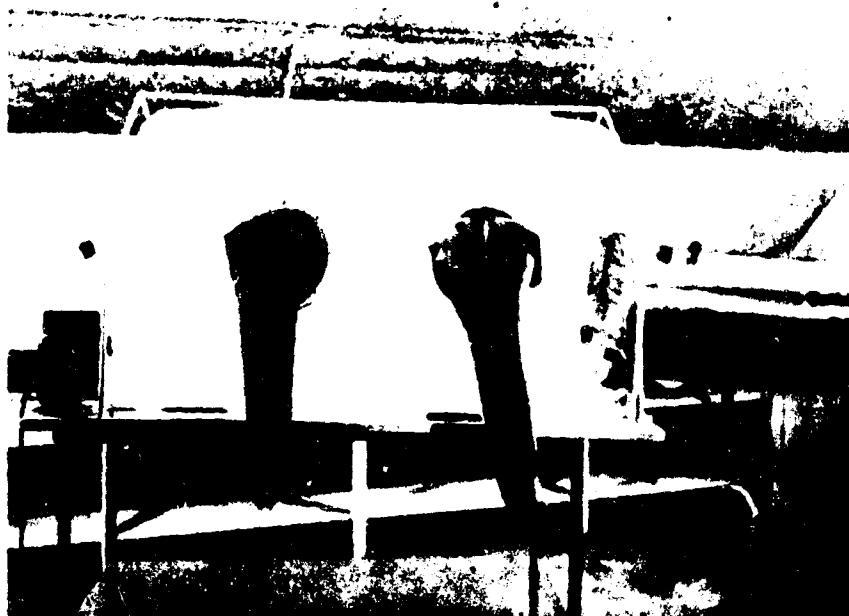(FD Neg B-8498)

Figure 3.   Plastic Isolator for Germ-Free Animals.



Figure 4.   Small Plastic Cabinet or Binocular Microscope.
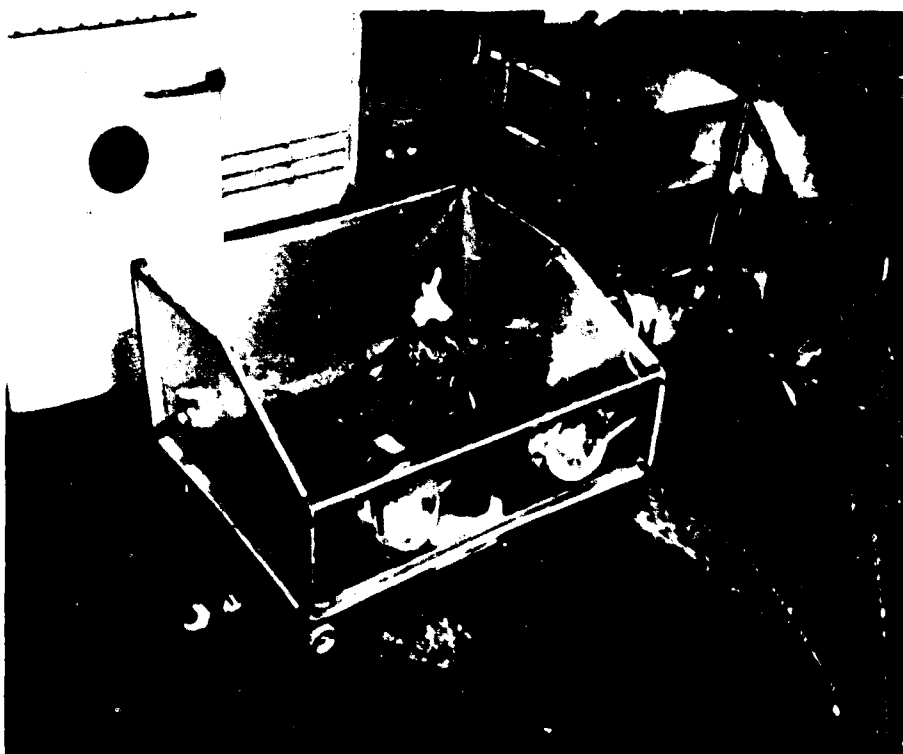            (FD Neg C-1262)

Figure 5.    Microscope Cabinet of Flexible Plastic Attached to a
Stainless Steel Cabinet.    (FD Neg C-2765)



Figure 6.    Plastic Enclosure for an Animal
Cage Rack.    (FD Neg C-1162)

Figure 7.   "Life-Island" Isolator for a Hospital Patient.



Figure 8.   Cabinet Made of Rigid Plastic Material.
(FD Neg C-2444)

Figures 9 through 18 show examples of various types of stainless steel
enclosures. Figure 9 shows the simplest type of microbiological safety
cabinet used in infectious disease laboratories. An inward flow of air
sweeps hazardous materials away from the operator and onto the exhaust
filter located above the cabinet. The same type of cabinet can be used
with out-flowing air in product protection systems.

Figure 10 shows a similar cabinet with the panel closed and arm-length
gloves attached to the glove ports. In this case an ultraviolet airlock is
provided on the right side for passage of materials. Cabinets of this
design can be used for personnel protection or product protection but they
are not considered absolute barriers because they are not constructed to
be gastight.

Figure 11 illustrates that cabinets of this type can be adapted to many
types of operations. In this case a microbiological barrier cabinet has
been built onto a refrigerated centrifuge.

For absolute microbiological barrier requirements, that is, when com-
plete containment or sterility must be preserved within a barrier, closed
and gastight cabinet systems are often required. Figure 12 shows an
example of a small sealed unit designed for one specific operation. This
unit can be presterilized and a single operation done under maximum
barrier conditions. Units of this size, however, are not particularly
efficient because of the limited amount of working space within them.

Figure 13 is a stainless steel germ-free chamber used in Lund, Sweden
by Gustafsson.

Figure 14 shows a Reyniers-type germ-free animal chamber in use at the
National Institutes of Health.

Figure 15 shows a line of gastight cabinets that will accommodate seven
operators. Notice that a continuous belt is provided in the cabinets for
movement of materials.

Figure 16 shows a larger gastight cabinet system arranged in a U shape
around three walls of a laboratory room. Each leg of the system terminates
in an autoclave. Disinfectant dunk baths for passage of materials into the
cabinet system are also provided.

Figure 17 shows a larger gastight cabinet system. This system has an
autoclave, a dunk bath, and an endless belt for movement of materials within
the system.

Figure 18 perhaps illustrates the maximum in complexity that has been
achieved up to now with gastight cabinet systems. The system shown here
contains working space for laboratory operations including enclosed incu-
bators, refrigerators, deep freezes, etc., and attached autoclaves and
disinfectant dunk baths, as well as cabinets at three levels for housing
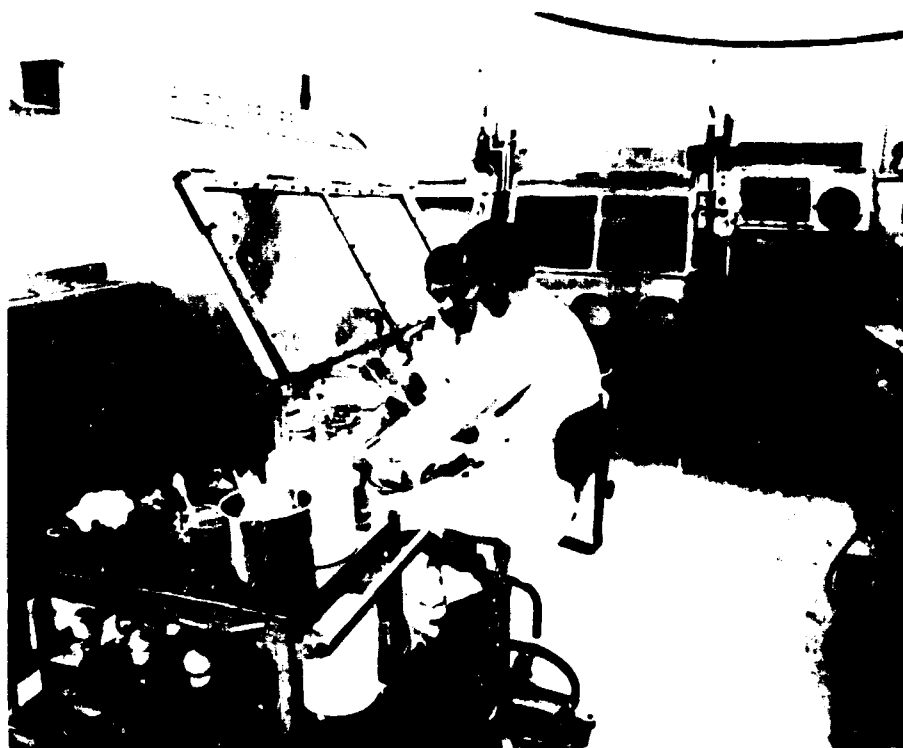animals being used in infectious disease research.

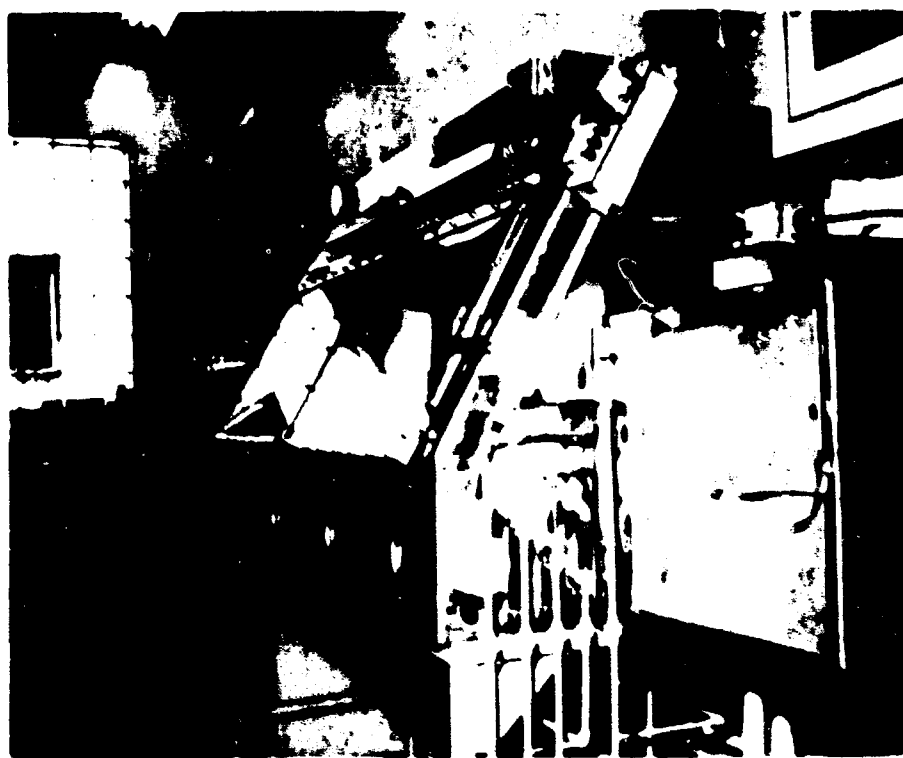Figure 9.  Open Panel Microbiological Safety Cabinets.



Figure 10.  Microbiological Safety Cabinet with Glove Panel
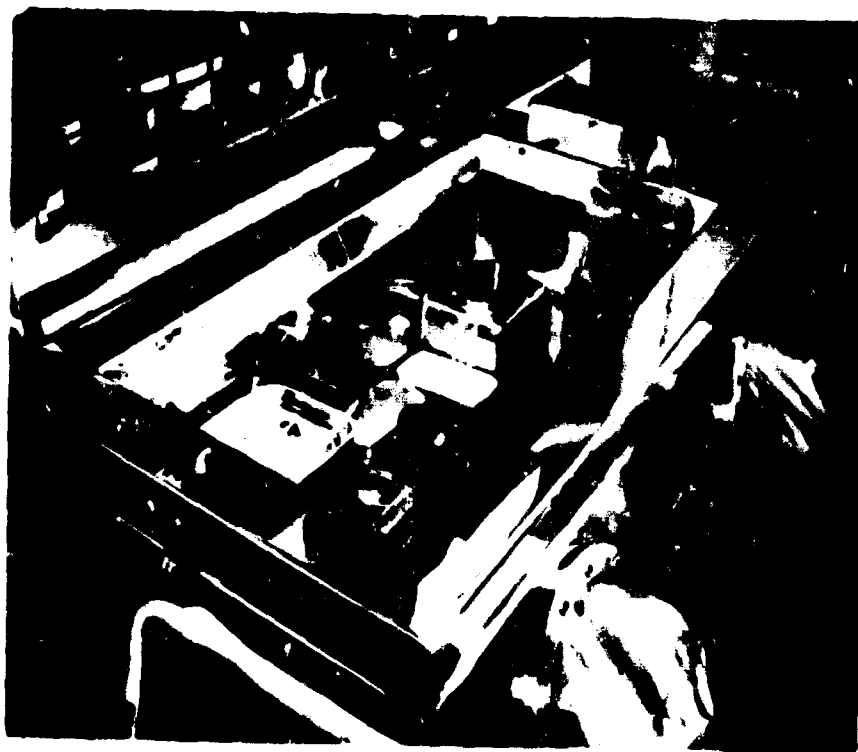and Gloves Attached.  (FD Neg C-4172)

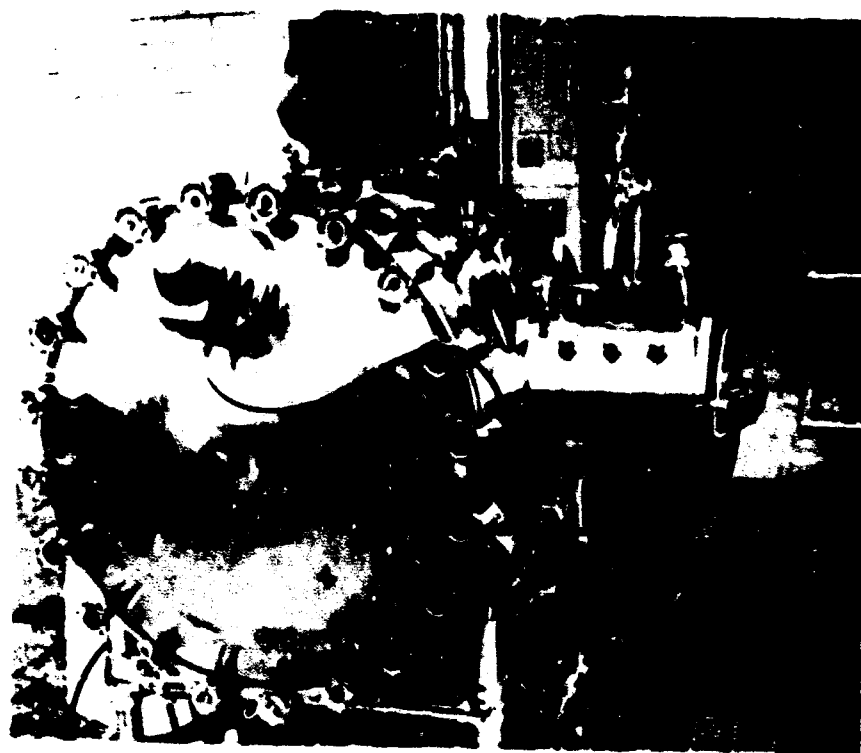Figure 13.   Gustafsson Germ-Free Animal Chamber.



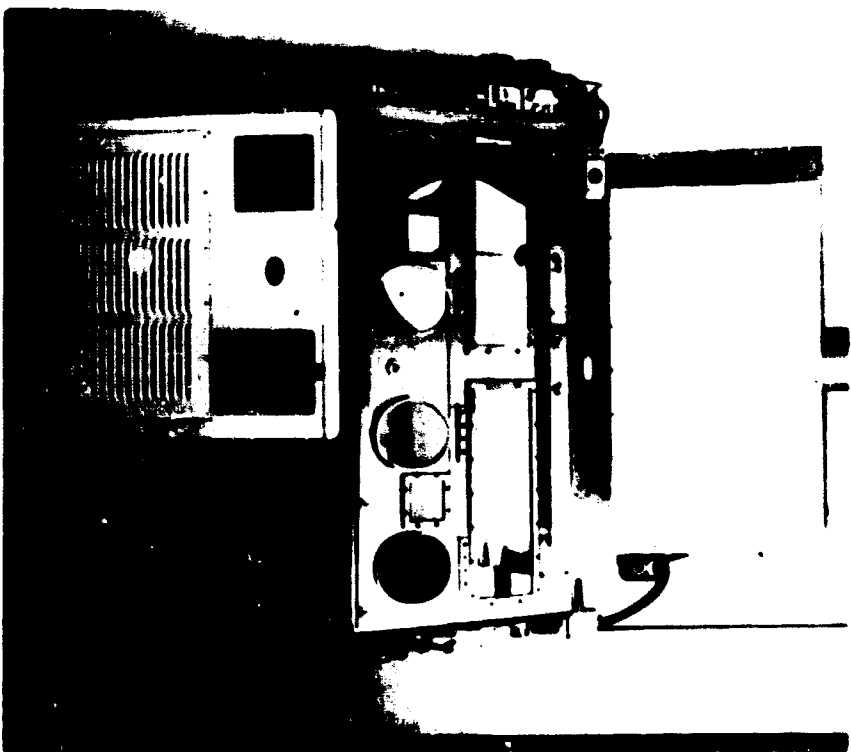Figure 14.   Reyniers-Type Germ-Free Animal Chamber.

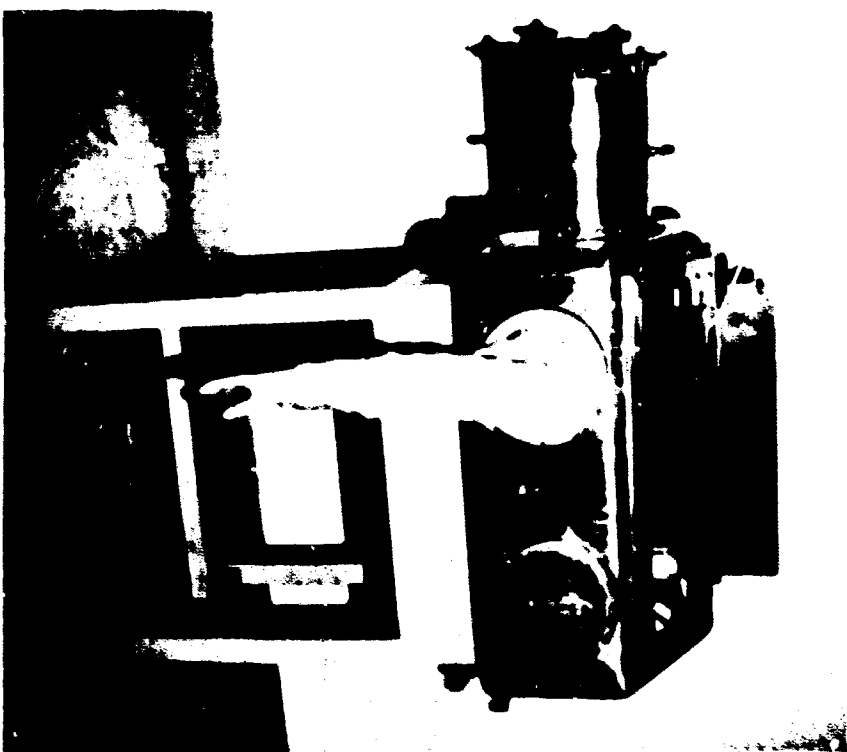Figure 11. Microbiological Cabinet for Centrifuge Operations. (FD Neg B-9317)



Figure 12. Small Gastight Microbiological Barrier. (FD Neg B-3868)

Figure 15. Gastight Cabinet System with Endless Belt.
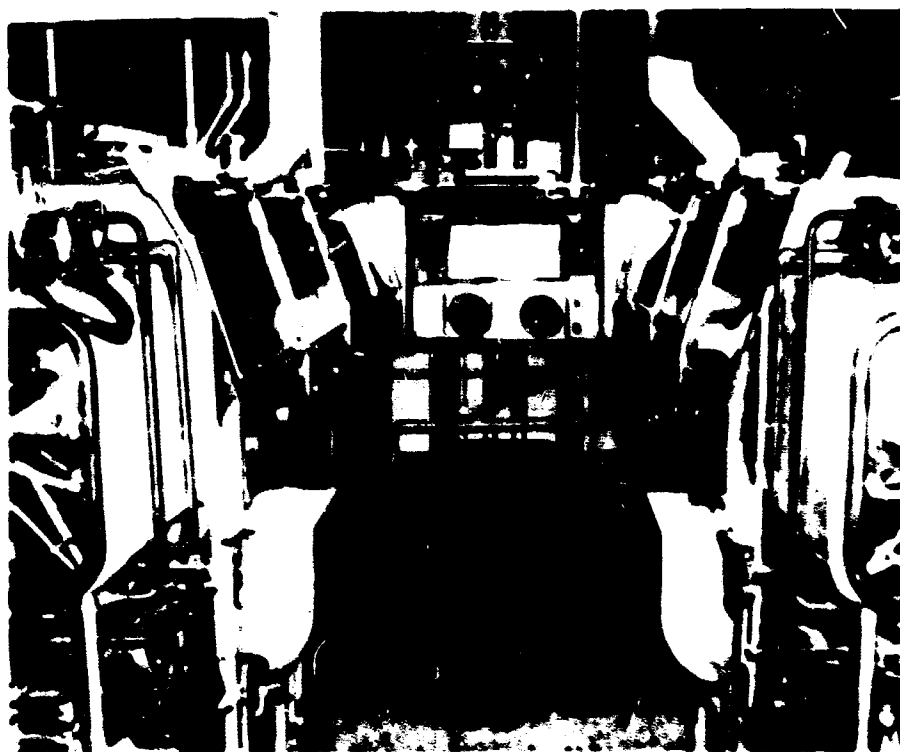(FD Neg B-6911)



Figure 16. Gastight Cabinet System with Autoclaves
and Dunk Baths. (FD Neg C-5120)

Figure 17. Large Gastight Cabinet System for Laboratory
Operations. (FD Neg C-5360)



Figure 18. Large Gastight Cabinet System for Laboratory
and Animal Research. (FD Neg C-4911)

Figure 19 shows one type of ventilated suit adaptable for use in sterile assembly rooms. The external surface of the suit can be sterilized with peracetic acid as the man enters a previously sterilized room. The air supply and air exhaust lines to the suits have to be regulated to maintain a negative pressure inside the suit in relation to the room. For personnel protection applications a positive pressure should be maintained within the suit. As with the flexible plastic cabinets, a major disadvantage of this type of system is the easy rupture of the suit during use.



Figure 19. Ventilated Suit. (FD Neg B-8216)

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**13 ABSTRACT**

Microbiological barriers prevent the migration of microbial contaminants. Historically, the use of barriers in laboratory operations was documented as early as in the 19th century. In relation to the steps normally taken to detect and control microbiological contamination, the tests used with microbiological barrier systems include air sampling, surface sampling, filter and air incinerator testing, and gas-tightness testing.

Microbiological barrier systems can be classified according to purpose, size, and degree of containment. Sterilization and decontamination agents are used with barrier systems for initial or terminal treatment, for the treatment of supplies and equipment moved in or out of the system, and for the maintenance of its microbiological state during use.

Irrespective of the shape and material used for microbiological cabinet barriers, there are certain desirable minimum features. Photographs of a number of present day microbiological barriers and barrier systems are presented.

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